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Abstract: Recent research suggests an important role of FKBP5, a glucocorticoid receptor regulating co-chaperone, in the development of stress-related diseases such as depression and anxiety disorders. The present study aimed to replicate and extend previous evidence indicating that FKBP5 polymorphisms moderate hypothalamus-pituitary-adrenal (HPA) function by examining whether FKBP5 rs1360780 genotype and different measures of childhood adversity interact to predict stress-induced cortisol secretion. At age 19 years, 195 young adults (90 males, 105 females) participating in an epidemiological cohort study completed the Trier Social Stress Test (TSST) to assess cortisol stress responsiveness and were genotyped for the FKBP5 rs1360780. Childhood adversity was assessed using the Childhood Trauma Questionnaire (CTQ) and by a standardized parent interview yielding an index of family adversity. A significant interaction between genotype and childhood adversity on cortisol response to stress was demonstrated for exposure to childhood maltreatment as assessed by retrospective self-report (CTQ), but not for prospectively ascertained objective family adversity. Severity of childhood maltreatment was significantly associated with attenuated cortisol levels among carriers of the rs1360780 CC genotype, while no such effect emerged in carriers of the T allele. These findings point towards the functional involvement of FKBP5 in long-term alterations of neuroendocrine stress regulation related to childhood maltreatment, which have been suggested to represent a premorbid risk or resilience factor in the context of stress-related disorders.

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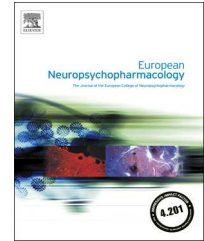


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Moderating role of *FKBP5* genotype in the impact of childhood adversity on cortisol stress response during adulthood

Arlette F. Buchmann^a, Nathalie Holz^a, Regina Boecker^a,
Dorothea Blomeyer^a, Marcella Rietschel^b, Stephanie H. Witt^b,
Martin H. Schmidt^a, Günter Esser^c, Tobias Banaschewski^a,
Daniel Brandeis^{a,d}, Ulrich S. Zimmermann^e, Manfred Laucht^{a,c,*}

^aDepartment of Child and Adolescent Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, J 5, 68159 Mannheim, Germany

^bDepartment of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Germany

^cDepartment of Psychology, University of Potsdam, Germany

^dDepartment of Child and Adolescent Psychiatry, Center for Integrative Human Physiology, Neuroscience Center Zurich, University of Zurich and ETH Zurich, Switzerland

^eDepartment of Psychiatry and Psychotherapy, University Hospital Carl Gustav Carus, Dresden, Germany

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Abstract

Recent research suggests an important role of *FKBP5*, a glucocorticoid receptor regulating co-chaperone, in the development of stress-related diseases such as depression and anxiety disorders. The present study aimed to replicate and extend previous evidence indicating that *FKBP5* polymorphisms moderate hypothalamus-pituitary-adrenal (HPA) function by examining whether *FKBP5* rs1360780 genotype and different measures of childhood adversity interact to predict stress-induced cortisol secretion. At age 19 years, 195 young adults (90 males, 105 females) participating in an epidemiological cohort study completed the Trier Social Stress Test (TSST) to assess cortisol stress responsiveness and were genotyped for the *FKBP5* rs1360780. Childhood adversity was assessed using the Childhood Trauma Questionnaire (CTQ) and by a standardized parent interview yielding an index of family adversity. A significant interaction between genotype and childhood adversity on cortisol response to stress was demonstrated for exposure to childhood maltreatment as assessed by retrospective self-report (CTQ), but not for prospectively ascertained objective family adversity. Severity of childhood maltreatment was significantly associated with attenuated cortisol levels among carriers of the rs1360780 CC genotype, while no such effect emerged in carriers of the T allele. These findings point towards the functional involvement of *FKBP5* in long-term alterations of neuroendocrine stress

*Corresponding author at: Department of Child and Adolescent Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, J 5, 68159 Mannheim, Germany. Tel.: +49 621 1703 4903; fax: +49 621 1703 1205.

E-mail address: manfred.laucht@zi-mannheim.de (M. Laucht).

regulation related to childhood maltreatment, which have been suggested to represent a premorbid risk or resilience factor in the context of stress-related disorders.

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1. Introduction

Stress-related psychiatric diseases such as mood and anxiety disorders are the most common mental health disorders in Western countries, with estimated 12-month prevalence rates of up to 14% in Europe (Wittchen et al., 2011). The identification of factors underlying individual differences in stress reactivity promises to improve the understanding and treatment of such disorders. One important neurobiological system activated by stress is the hypothalamus-pituitary-adrenal system (HPA axis). The magnitude and duration of the endocrine stress response are, in part, regulated by the negative feedback of cortisol on the HPA axis activity. Cortisol binds to glucocorticoid receptors (GR) at the level of the hypothalamus and the pituitary, resulting in an inhibition of ongoing CRH and ACTH release. Disturbances of the HPA axis have been repeatedly implicated as markers of several diseases, such as depression or Posttraumatic Stress Disorder (PTSD). Moreover, individual variation in neuroendocrine stress regulation has been suggested to represent a premorbid risk or resilience factor in the context of stress-related disorders (Binder and Holsboer, 2012).

There is ample evidence of genetic variation affecting GR-mediated regulation of the HPA axis. Different GR gene variants have been identified as affecting the cortisol reactivity (Wüst et al., 2004; Derijk and de Kloet, 2008). Growing evidence emphasizes polymorphisms of the gene coding for FKBP5-binding protein 51 (FKBP5), a regulator of GR function, as important determinants of HPA axis functioning (Velders et al., 2011; Ising et al., 2008). The most frequently investigated single nucleotide polymorphism (SNP) of the *FKBP5* gene is rs1360780, for which a functional effect towards increased FKBP5 protein levels was first suggested by Binder et al. (2004). In lymphocytes of carriers of the TT genotype, FKBP5 levels were observed to be twice as high as in C-allele carriers. As FKBP5 is a co-chaperone reducing the binding affinity of the GR (Denny et al., 2000), its overexpression would be expected to result in attenuation of the negative feedback inhibition. Accordingly, Ising et al. (2008) reported impaired cortisol recovery after stress among carriers of the minor T allele. Likewise, Luijk et al. (2010) observed increased cortisol reactivity during a stressful separation procedure in 14-month-old infants carrying the T allele.

Consistent with the assumption that neurobiological consequences of elevated cortisol exposure in brain areas related to emotional processing may contribute to the development of mood disorders (Cowen, 2010), rs1360780 has also been linked to major depression risk and antidepressant treatment response (Zimmermann et al., 2011; Binder et al., 2004). Notably, a moderating role of this polymorphism in the vulnerability to stress-related disorders in the presence of environmental adversity was documented. When exposed to adverse, especially traumatic events, homozygous carriers of

the minor T allele were found to be at increased risk of depression compared to other genotype groups (Zimmermann et al., 2011; Appel et al., 2011). The same genotype has also been reported to predict the severity of posttraumatic stress symptoms after exposure to childhood adversity, suggesting that FKBP5-mediated alteration of GR sensitivity may serve as a potential risk factor for PTSD in the face of traumatic stress (Binder et al., 2008; Xie et al., 2010). Further support that genetic variation in *FKBP5* may account for an increased vulnerability to psychopathology has been provided by pre-clinical research. When comparing wild-type mice with *FKBP5* knockout animals, O'Leary et al. (2011) reported that the deletion was associated with less corticosterone production after stress and an increased resilience to depression-like behavior.

Most recently, Klengel et al. (2013) suggested that epigenetic modifications constitute a molecular mechanism which may account for dysregulation of the stress hormone system and increased vulnerability to stress-related disorders in rs1360780 T-allele carriers. Genotype-dependent differences in DNA methylation in functional glucocorticoid response elements (GRE) of *FKBP5* were linked to GR resistance and were hypothesized to induce long-term dysregulation of the endocrine stress system among individuals who had been exposed to childhood trauma.

Using data from a prospective study starting at birth, we aimed to (i) replicate the finding from previous studies suggesting a functional involvement of the *FKBP5* rs1360780 polymorphism in regulating the HPA axis response to stress and (ii) examine whether the recently reported long-term epigenetic changes resulting from the interaction of *FKBP5* genotype with adverse childhood experiences are reflected in the individuals' cortisol response to acute stress as supposed by Klengel et al. (2013). Moreover, the present study contributes to and extends the existing literature by providing measures of childhood adversity drawn from both retrospective self-report and prospectively ascertained objective information collected in the same sample.

2. Experimental procedures

2.1. Sample

This investigation was conducted in the framework of the Mannheim Study of Children at Risk, an ongoing epidemiological cohort study of the long-term outcome of early risk factors (Laucht et al., 1997; Laucht et al., 2000). Assessments were conducted at the age of 3 months and at regular intervals throughout development until young adulthood. The present analysis comprises 195 young adults (90 males, 105 females) who participated in the 19-year assessment, including a laboratory session with a standardized psychosocial stress protocol, the Trier Social Stress Test (TSST, Kirschbaum et al., 1993), for whom all relevant information was available. Exclusion criteria included pregnancy, potentially adverse effects in

participants suffering from acute psychiatric symptoms, alcohol/drug dependence or abuse, positive urine drug screening, missing or pathological hormone data, treatment with glucocorticoids and nausea during vein puncture. The study was approved by the ethics committee of the University of Heidelberg and written informed consent was obtained from all participants.

2.2. Psychological assessments

The Structured Clinical Interview for DSM-IV (SCID-I German version (Wittchen et al., 1997)) was conducted at the age of 19 years to assess psychiatric disorders in the young adults. To examine current drug use, a substance use inventory was administered (Müller and Abbet, 1991). On the day of the experiment, participants were asked about any use of current medication. In addition, female participants reported their menstrual cycle phase and any use of contraceptive medications.

Childhood adversity was assessed in two ways. On the one hand, childhood maltreatment was retrospectively assessed with the brief screening version of the Childhood Trauma Questionnaire (CTQ, Bernstein et al., 2003) at the age of 23 years. The CTQ is a self-report inventory distinguishing five types of *childhood maltreatment*, i.e. sexual, physical, and emotional abuse, and emotional and physical neglect. Good psychometric properties have been confirmed for the German version (Wingenfeld et al., 2010). The CTQ yields a total score and subscale scores for each type of maltreatment. Means, standard deviations, and ranges of the CTQ scales in the present sample are given in Table 1. In order to avoid using cut-offs and categorization, which, in the present sample, would result in low absolute numbers of individuals reporting a history of severe maltreatment, the total score was used as a continuous measure of individual history of maltreatment.

On the other hand, exposure to childhood adversity was determined according to an 'enriched' *family adversity* index as proposed by Rutter and Quinton (1977). Information was derived from a standardized parent interview conducted by trained interviewers at each assessment during childhood (age 3 months; 2; 4.5; 8 and 11 years). The index measures the presence of 11 adverse family factors, covering characteristics of the parents (e.g., psychiatric disorder, low educational level), the partnership (e.g., marital discord, early parenthood), and the family environment (e.g., poor social integration, overcrowding) during a period of 1 year prior to the assessment (Laucht et al., 1997, 2012). A total family adversity score was calculated by counting the number of factors present in the period until 11 years of age. The correlations between the family adversity score and CTQ measures are shown in Table 1.

2.3. Trier Social Stress Test (TSST)

Participants arrived at the laboratory at 13:00. First, all subjects were fully informed about the study procedures, including the requirement to abstain from nicotine and caloric intake throughout the experiment. Urine was screened for cannabinoids, opioids, cocaine, methamphetamine, benzodiazepines, and amphetamines using standard qualitative immunological methods (MAHSAN Diagnostika, Reinbek, Germany). A venous catheter was inserted for blood sampling at 13:30. All subjects underwent a urine drug screening. At 14:40, participants were introduced to the stress test procedure, and this time was defined as the onset of stress exposure. They were then given 10 min to prepare an oral presentation as for a job interview. After this, they performed their presentation and a mental arithmetic task in front of three researchers acting as an audience (Kirschbaum et al., 1993). Blood samples were obtained before the introduction to the TSST and immediately before and 10, 20, 35, 50, and 70 min after the beginning of the speech. Cortisol analysis was performed as described previously (Witt et al., 2011).

2.4. Genotyping

Genomic DNA was prepared from whole blood or saliva according to standard procedures. Rs1360780 was genotyped using a pre-designed TaqMan 5' nuclease SNP genotyping assay (Life Technologies, USA; assay number: C_8852038_10). Accuracy was assessed by duplicating 15% of the original sample, yielding a reproducibility of 100%.

2.5. Statistical analysis

To examine genotype differences with regard to sex, smoking status, female menstrual cycle phase, oral contraceptive use, family adversity and childhood maltreatment, χ^2 tests and ANOVAs were conducted as appropriate. For the analysis of hormonal responses, cortisol measures were transformed to the logarithmic scale due to non-normality. First, a repeated measures ANCOVA design was utilized to assess the effect of time, genotype and their interactions on the hormonal response to the TSST. Cortisol variation was investigated until 70 min after the beginning of the speech (7 assessments). Time after stress onset was applied as a within-subject factor and genotype was included as a between-subject factor. To examine intraindividual cortisol change, in a second set of analyses, individual delta scores were formed, defined as the difference between the individual peak (in the period 35 min after cessation of the TSST) and baseline hormone level (immediately before the introduction to the TSST). Post-hoc univariate ANCOVAs were performed to examine the impact of genotype on baseline hormone level, delta variation and cortisol recovery (70 min after the beginning of the speech). In a third step, the same analyses were conducted, including family adversity and childhood maltreatment, respectively, and their interaction with genotype as predictors of the individual cortisol response to stress. Both measurements of childhood adversity were treated as continuous variables. To control for the influence of gender, smoking status, use of oral contraceptives and baseline cortisol level on the hormonal stress response, these factors were included as covariates in the analyses. Greenhouse-Geisser corrections were applied where appropriate, and only adjusted results are reported.

3. Results

3.1. Sample characteristics

After excluding 45 individuals for reasons given in Table 2, the final sample included $N=195$ individuals. FKBP5 rs1360780 genotype frequencies were CC=94; CT=84 and TT=17, which was in accordance with Hardy-Weinberg equilibrium ($\chi^2=.08$, $p=.773$). Of the females, 70.5% were using oral contraceptives (OC) and 47.6% were in the luteal phase of their menstrual cycle. Information on menstrual cycle phase was not available in 9.5%. Genotype groups did not significantly differ with respect to gender, use of oral contraceptives, menstrual cycle phase (all $\chi^2<3.10$; $p>.307$), family adversity [$F(2, 192)=.40$, $p=.672$] or childhood maltreatment [$F=.07(2, 192)$, $p=.929$]. However, there was a trend towards a lower number of daily smokers in the CT genotype group ($\chi^2=4.97$; $p=.083$, Table 2).

3.2. Effect of FKBP5 polymorphism

Significant changes in plasma cortisol emerged due to stress exposure, with an increase following the TSST procedure and a decrease after stress cessation [main effect of time: F

(6, 1134)=83.16, $p < .001$]. In addition, a significant time by genotype interaction was observed [$F(12,1134)=2.93$, $p=.008$; see Figure 1], indicating that carriers of the rs1360780 T allele displayed a more pronounced cortisol response to the TSST than homozygous carriers of the C allele. Post-hoc analyses revealed that the pre-stress plasma cortisol concentration and the individual cortisol increase in response to the TSST (delta) were unaffected by genotype [baseline: $F(2,189)=1.20$, $p=.30$; delta: $F(2, 189)=.39$, $p=.676$]. However, significant group differences emerged regarding cortisol recovery after completion of the stress test [$F(2,188)=3.63$, $p=.028$]. When cortisol was measured 70 min after speech onset, carriers of the CC genotype showed significantly lower cortisol levels than CT ($p=.027$) and TT ($p=.042$) carriers, while no difference in cortisol recovery was observed among the latter groups (CT vs. TT: $p=.451$).

3.3. Effect of gene-environment interaction

Based on these results and due to the small number of T homozygotes, individuals carrying the CT or TT genotypes were combined into one group in the subsequent analyses examining gene-environment interaction effects. While there was no significant three-way interaction of genotype

by family adversity by time [$F(6,1128)=.458$, $p=.713$], repeated measures ANCOVA revealed a significant three-way interaction of *FKBP5* genotype by childhood maltreatment by time to predict cortisol stress response [$F(6,1128)=2.629$, $p=.049$; Figure 2]. Post-hoc analyses revealed that the impact of exposure to childhood maltreatment on cortisol recovery was significantly moderated by *FKBP5* genotype [interaction genotype by maltreatment $F(1,187)=4.65$, $p=.032$]. However, neither pre-stress plasma cortisol concentration nor delta cortisol was affected by the interaction between childhood maltreatment and genotype. In homozygote carriers of the C allele, severity of childhood maltreatment was significantly associated with decreasing overall cortisol levels [$F(1,89)=5.23$, $p=.008$] and faster cortisol recovery when assessed 70 min after speech onset [$F(1, 88)=10.29$, $p=.002$]. In contrast, childhood maltreatment was unrelated to cortisol stress reactivity [$F(1,96)=.01$, $p=.947$] and cortisol recovery [$F(1,95)=.03$, $p=.856$] in carriers of the T allele.

4. Discussion

In accordance with previous research (Ising et al., 2008; Luijk et al., 2010), the T allele of the *FKBP5* rs1360780 polymorphism was found to be associated with delayed cortisol recovery

Table 1 Means, standard deviations (SD), and ranges of the childhood adversity measures in the study sample ($N=195$).

CTQ scale	Mean (SD)	Range	N (%) above the threshold for			Correlation with family adversity score
			Low	Moderate	Severe	
Total score	30.68 (7.56)	25-87				.272*
Emotional abuse	6.48 (2.83)	5-25	18 (9.2)	6 (3.1)	3 (1.5)	.162*
Physical abuse	5.41 (1.60)	5-22	6 (3.1)	3 (1.5)	1 (.5)	.153*
Sexual abuse	5.14 (.76)	5-12	6 (3.1)	2 (1.0)	0 (0)	.052
Emotional neglect	7.95 (3.17)	5-24	39 (20.0)	6 (3.1)	2 (1.0)	.292*
Physical neglect	5.70 (1.33)	5-12	10 (5.1)	7 (3.6)	0 (0)	.291*
Family adversity score	3.65 (2.43)	0-10				

CTQ=Childhood Trauma Questionnaire

* $p < .05$

Table 2 Sample characteristics by *FKBP5* rs1360780 genotype.

<i>FKBP5</i> rs1360780 genotype	CC	CT	TT
TSST sample ($N=240$)	116	102	22
Excluded for (N)			
Positive drug screening	8	4	2
DSM IV substance use disorder	5	2	1
Missing or implausible cortisol data	9	12	2
Evaluated ($N=195$)	94	84	17
Males N (%)	45 (47.9)	38 (45.2)	7 (41.2)
Daily smokers N (%)	31 (33.0)	16 (19.0)	6 (35.3)
Females with oral contraceptive use N (%)	31 (63.3)	35 (76.1)	8 (80.0)
Family adversity score M (SD)	1.86 (1.95)	1.61 (1.84)	1.71 (1.93)
CTQ total score M (SD)	30.53 (8.13)	30.71 (7.15)	31.29 (6.65)

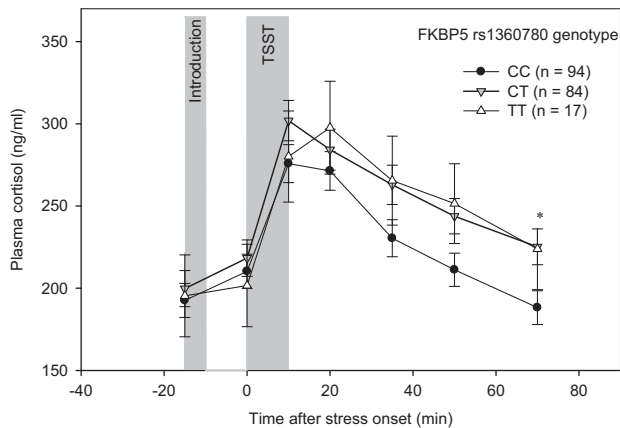


Figure 1 Cortisol responses to the TSST depending on *FKBP5* rs1360780 genotype. Error bars represent SE (*significant group difference, $p < .05$).

after exposure to acute psychosocial stress. Enhanced induction of *FKBP5* mRNA in association with this polymorphism was suggested to interfere with the GR-mediated negative feedback (Binder et al., 2004), thereby predisposing T-allele carriers to HPA dysregulation typical for stress-related disorders. In particular, when exposed to childhood adversity, an increased risk of psychiatric disease has been documented among risk allele carriers. However, to the best of our knowledge, this is the first study to demonstrate *FKBP5*-dependent long-term effects of childhood maltreatment on the hormonal response to stress.

In contrast to a previous investigation reporting an additive effect of *FKBP5* rs1360780 and environmental adversity in predicting cortisol reactivity in infants (Luijk et al., 2010), a significant interaction of genotype with childhood maltreatment emerged in the current study. Our results indicated faster cortisol recovery after acute stress in young adults with the CC genotype who had been exposed to maltreatment during childhood, whereas no significant association of childhood maltreatment with the cortisol stress response was observed among T-allele carriers. Several reasons may account for the discrepant findings, including age-dependent effects and methodological differences in stress induction and cortisol sampling. Moreover, insecure-resistant attachment was considered as an indirect marker of environmental adversity during infancy by Luijk et al. (2010), whereas the present study compared two different measures of childhood adversity. Gene-environment interaction related to cortisol stress response was demonstrated for exposure to childhood maltreatment as assessed by retrospective self-report, but not for a prospectively ascertained objective measure of adversity.

This finding is in line with previous evidence suggesting that the CTQ may identify individuals who are genetically susceptible to stress-related disorders due to increased consolidation of memories of emotionally arousing experiences (Polanczyk et al., 2009). On the one hand, the family adversity index used here has the advantage of being largely independent of memory effects of the participants, as it was assessed by trained interviewers in repeatedly conducted parent interviews. Therefore, it might include information that was not recalled by the young adults. On the other hand, we cannot rule out that the absence of an

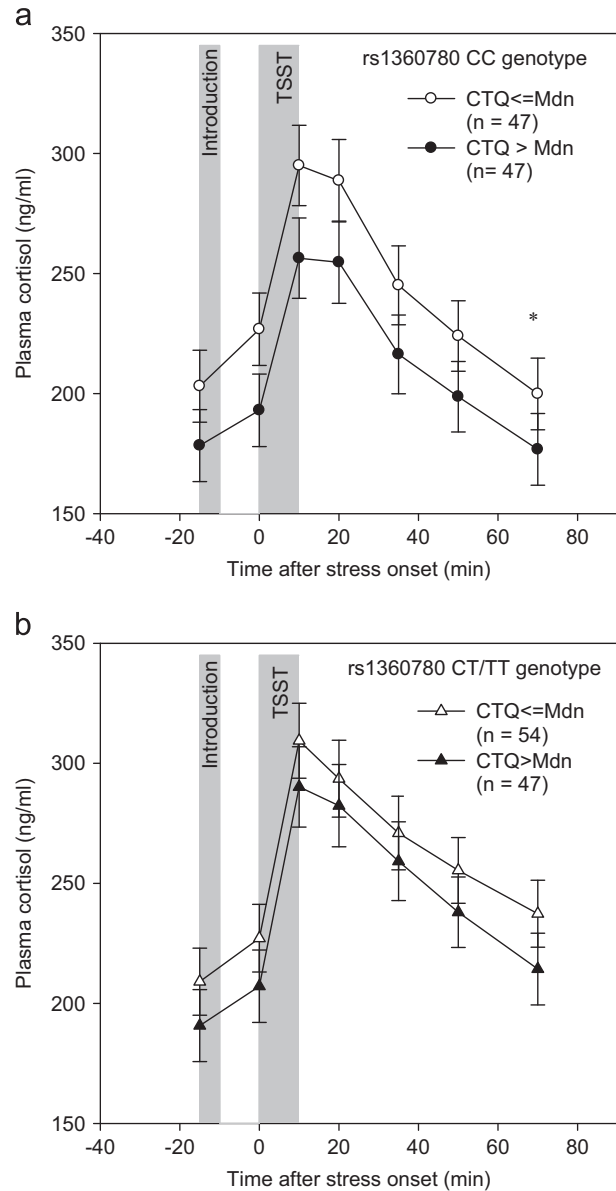


Figure 2 Cortisol responses to the TSST depending on the interaction between *FKBP5* rs1360780 genotype and exposure to childhood maltreatment (CTQ total). Median split of the CTQ was used to visualize the gene by environment interaction (Mdn=28). While a higher level of maltreatment was associated with significantly reduced cortisol levels in (a) *FKBP5* rs1360780 CC carriers, the cortisol stress response was unaffected in carriers of the (b) *FKBP5* rs1360780 T allele. Error bars represent SE (*significant group difference, $p < .05$).

rs1360780 by family adversity interaction is due to the fact that this measure covers qualitatively different stressors than the CTQ. In particular, the family adversity index did not address childhood maltreatment per se but rather pertained to a more broader spectrum of adverse conditions such as marital discord or parental psychiatric illness (see also Supplementary Table 1). Thus, the moderating effect of *FKBP5* might be specific to the type of maltreatment experienced (emotional maltreatment, neglect, physical abuse or sexual abuse as assessed by the CTQ). However,

epidemiological surveys documenting the detrimental impact of adverse experiences during on mental health have suggested that the risk of psychiatric disorders increased with the number of multiple adversities indicative of maladaptive family functioning which were not limited to physical and sexual abuse or neglect (Green et al., 2010; Kessler et al., 2012).

Recently, Klengel et al. (2013) identified a molecular mechanism which they suggested as explaining a genotype-moderated effect of exposure to childhood adversity on stress hormone system regulation. The authors reported significant variation of DNA methylation of the *FKBP5* locus according to rs1360780 genotype. While childhood maltreatment proved to be associated with *FKBP5* demethylation in carriers of the T allele, no such effect was observed among C homozygotes. Such epigenetic-induced changes result in higher *FKBP5* responsiveness, probably increasing the risk of dysregulation of the stress hormone system, such as in stress-related disorders. A delayed cortisol recovery may, therefore, be regarded as a prominent risk factor, predisposing *FKBP5* risk allele carriers to stress-related disease.

Surprisingly, the present study revealed no additional increase in cortisol levels among carriers of the *FKBP5* T allele due to exposure to childhood maltreatment. Instead, homozygous carriers of the C allele showed significant attenuation of the cortisol response to stress. Concerning the implications of childhood maltreatment for the hormonal stress system, disparate findings have been reported in the literature, including both hyper- and hyporeactivity. Prospective longitudinal research has indicated that adverse experiences in childhood initially lead to higher cortisol levels, which may become suppressed over time (Trickett et al., 2010). It was suggested that the attenuation of stress-regulatory responses may develop as an adaptive process to avoid chronic activation and to protect individuals experiencing childhood adversity against the deleterious effects of long-term exaggerated glucocorticoid exposure (Susman, 2006). Saxbe et al. (2012), for instance, reported that decreased cortisol reactivity during a conflict task was associated with lower psychological and behavioral problems among youths who grew up in aggressive family environments, whereas a more exaggerated cortisol reactivity appeared to be rather maladaptive in the context of an aggressive family environment. Thus, a blunted cortisol response to environmental challenges, as observed in rs1360780 CC carriers, may be viewed as indicative of an enhanced biological resilience in healthy individuals with a history of childhood adversity (Saxbe et al., 2012; Elzinga et al., 2008). Corresponding evidence was provided earlier on by animal studies in the framework of the stress-inoculation model, which assumes that stress exposure in early life may increase resilience to stressful events later in life (Parker and Maestriperi, 2011). Mechanisms responsible for this counterregulatory adaptation were assumed to include increased hippocampal GR expression (Kaffman and Meaney, 2007) or increased negative feedback sensitivity of the HPA system (Heim et al., 2000).

On the other hand, as cortisol hyposecretion has been linked to psychopathology, such as externalizing problems (Fairchild et al., 2008; Popma et al., 2006; van Goozen et al., 1998), posttraumatic stress disorder and other stress-related disorders (Heim et al., 2000), it could also

constitute a risk factor for the development of these disorders. Whether childhood adversity leads to risk or resilience is likely to be attributable to the type (e.g. family adversity vs. maltreatment) or the timing of exposure but also to moderating factors such as genotype. Emerging evidence has outlined the important role of *FKBP5* in the vulnerability to stress-related psychiatric disorders (Binder et al., 2008; Kang et al., 2012; Zimmermann et al., 2011). In accordance with this, the present results could be interpreted as suggesting that, instead of stress inoculation, T-allele carriers exposed to childhood maltreatment may exhibit a sensitization of emotion processing systems. An increased hormonal stress response may thus be accompanied by a heightened vigilance towards threatening information as suggested by Fani et al. (2013). These authors reported increased activation of the hippocampus and attentional bias to angry faces among traumatized carriers of the rs1360780 T allele. Correspondingly, the *FKBP5* genotype has been implicated in amygdala activation to threat-related stimuli in the context of childhood emotional neglect (White et al., 2012). While in TT carriers amygdala activity was shown to increase with the level of childhood neglect, no such effect was observed in CC carriers.

When evaluating the present findings, several issues have to be considered. First, the sample size of this study was relatively small for a genetic association study. Hence, the results have to be considered as exploratory in nature and should be replicated in independent samples. Second, since repeated exposure to the stress paradigm applied here could have resulted in a more valid estimate of the cortisol response pattern (Federenko et al., 2004), the contribution of *FKBP5* genotype to individual variation in cortisol reactivity was likely underestimated in the present study due to the use of a single TSST assessment. Third, the present investigation comprised a community sample of young adults characterized by low levels of exposure to severe childhood maltreatment, as assessed by the CTQ. This restriction clearly reduces the ability to generalize the present findings to other samples with higher exposure to childhood adversity, in particular physical or sexual abuse. Most previous studies relied on categorical measures using cut-offs to define severe cases of maltreatment. In contrast, the present investigation conducted in an epidemiological cohort sample used a continuous measure of individual history of childhood maltreatment, thus also including a greater number of mild cases. Therefore, the findings reported here may be interpreted as extending the current evidence of the *FKBP5* by childhood adversity interaction to a less narrow definition of maltreatment. Fourth, due to the low number of individuals exposed to severe maltreatment, the statistical power to address the research questions is limited. Given the finding by Klengel et al. (2013), one would clearly expect the abused homozygous T allele carriers to demonstrate higher and prolonged cortisol responses than the abused C allele carriers. However, in the present study, it remained unclear whether such an effect emerged, as CT and TT carriers had to be combined due to the small sample size.

In addition, the sample of this study was initially enriched with children who grew up in an environment characterized by several indicators of maladaptive family function which have been shown to be more frequently associated with

maltreatment than by random chance. Studies examining the effects of childhood adversity on HPA axis function in the long run have been confronted with the problem that, among participants exposed to high levels of adversity, the rate of individuals suffering from organic or psychiatric diseases (including intoxication with drugs of abuse or psychoactive medication) was enhanced, thereby increasing the risk of confounding the results. To demonstrate this problem in the present sample, significantly higher CTQ scores were obtained among participants excluded from the investigation for reasons given in Table 2 (CTQ total score: $M=34.24$, $SD=13.22$) as compared to the evaluated TSST sample ($F(1, 238)=5.90$, $p=.016$). Another shortcoming of the present study is that the participants completed the CTQ 4 years after their participation in the TSST, while psychopathology was not assessed in parallel. As Polanczyk et al. (2009) clearly noted, the CTQ taps depression-relevant emotional memories, therefore, we cannot rule out that the incidence of psychiatric disorders at the time of CTQ assessment may have affected the results.

To summarize, our results indicated that a genetic variant of *FKBP5* that has been repeatedly associated with an increased risk of depression and other stress-related disorders (Binder, 2009) moderated the long-term impact of childhood maltreatment on the hormonal stress regulation system. As GR-mediated feedback mechanisms have been suggested to play a major role in the pathophysiology of psychiatric disorders (Holsboer, 2000) and to affect antidepressant treatment (Binder, 2009), future clinical studies should investigate the significance of gene-environment interaction in predicting individual treatment responses.

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Contributors

A. Buchmann wrote the first draft of the manuscript, managed the literature searches and analyses together with N. Holz. D. Blomeyer and U.S. Zimmermann were responsible for the acquisition of data. M. Rietschel and S.H. Witt contributed to the molecular genetic analyses. R. Boecker, D. Brandeis and T. Banaschewski contributed to the analysis and interpretation of the data. M. Schmidt, G. Esser and contributed to the conception and funding of the study. M. Laucht designed the study and wrote the protocol. All authors contributed significantly to and have approved the final manuscript.

Conflict of interest

Dr. Banaschewski served in an advisory or consultancy role for Bristol Myers-Squibb, Desitin, Lilly, Medice, Novartis, Pfizer, Shire and Viforphan. He received conference attendance support and conference support or received speaker's fees from Lilly, Janssen McNeil, Medice, Novartis, Shire. He is/has been involved in clinical

trials conducted by Lilly, Shire and Novartis. Dr. Zimmermann's work has been funded by the BMBF, DFG, NIAAA. Since 2010, he has received compensation for educational talks and workshops from: Lundbeck, Gewerkschaft Erziehung und Wissenschaft, Sächsische Landesärztekammer, Park-Krankenhaus Leipzig, Servier, Janssen, GSK, Pfizer, Klinikum Chemnitz. The present work is unrelated to the above grants and relationships. All other authors declare no conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.euroneuro.2013.12.001>.

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